

## Evidence by Electron Micrographs for a High Incidence of Bacteriophage Particles in the Waters of Yaquina Bay, Oregon: Ecological and Taxonomical Implications†

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A variety of viral particles, the majority of them clearly identifiable as bacteriophages, were found in the seawater of Yaquina Bay, Oregon. These phages were obtained as free particles from the seawater without employing specific hosts for enrichments or further purification in the laboratory. A variety of electron micrographs showing different morphologies of phages as well as phage-bacterium interactions found in the seawater are presented. In the area where the bay received organic enrichment from seafood processing plants, a minimum of  $10^4$  phage particles per ml was estimated. Since the technique used was designed to concentrate particles  $0.2\ \mu\text{m}$  in diameter or larger, it is assumed that the actual number of phage particles is higher than  $10^4$  particles per ml. The implications of the presence of such phage concentrations in bays and estuaries with a certain level of eutrophication are of obvious importance in considering the microbial ecology of these environments.

Although the occurrence of bacteriophages in nature is well known, our knowledge of the numbers of phages in nature leaves much to be desired. The major emphasis has been on quantifying the coliphages in sewage or sewage-contaminated water (7, 12). Various investigators (3, 8, 14) have isolated bacteriophages from the marine environment by employing the enrichment technique. In some instances direct counts of phage for a specific host gave positive results (9, 14). Because of the data presented in the literature, which indicate the paucity of phages in the marine environment, bacteriophages have not been considered to play a significant role in the dynamics (both genetic and demographic) of the bacterial population. Recently, Johnson and Sieburth (Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, N95, p. 178) reported that the microscopic observation of bacteriophages in offshore waters and bays is not a rare event. This paper presents semiquantitative evidence from electron microscopy for the high incidence of viral particles in the seawater of Yaquina Bay, Oregon.

### MATERIALS AND METHODS

**Treatment of samples.** Because the primary purpose of this investigation was the study of the natural

bacterial population in Yaquina Bay, membrane filters ( $0.2\text{-}\mu\text{m}$  pore size; Nucleopore Corp.) were used for filtration and concentration of samples; free viral particles smaller than  $0.2\ \mu\text{m}$  would tend to be lost during the filtration process.

Water samples were collected in various areas of the bay, kept on ice, concentrated by filtration, and fixed for electron microscopy within 60 min. Generally, 200 ml of seawater was filtered through a  $0.2\text{-}\mu\text{m}$  Nucleopore membrane filter to concentrate the phage particles on the filter. The phage particles on the filter were resuspended in 4 ml of the same water. In the area near the seafood processing plant the samples were prepared in the same manner, but the particles were suspended in 1/6 to 1/10 the volume of water filtered. No enrichment processes employing specific host bacteria were used in this investigation.

**Electron microscopy.** Concentrated samples were fixed with magnesium carbonate-neutralized glutaraldehyde (final concentration, 1%) for 12 h at  $4^\circ\text{C}$ . After fixation, 10 ml of distilled water was added to the sample, which was then filtered through a  $0.2\text{-}\mu\text{m}$  Nucleopore membrane filter. The filtered material was resuspended in distilled water. Uranyl acetate (0.5%) was used for positive and negative staining. Electron micrograph grids were covered with Formvar film and coated with carbon. Samples were observed with a Philips 300 electron microscope operating at 60 kV. Microscope magnifications were routinely calibrated with bovine catalase.

### RESULTS AND DISCUSSION

Figures 1 and 2 show a variety of the morphologies of the free viral particles found in the bay. Most of the particles shown in Fig. 1 and 2

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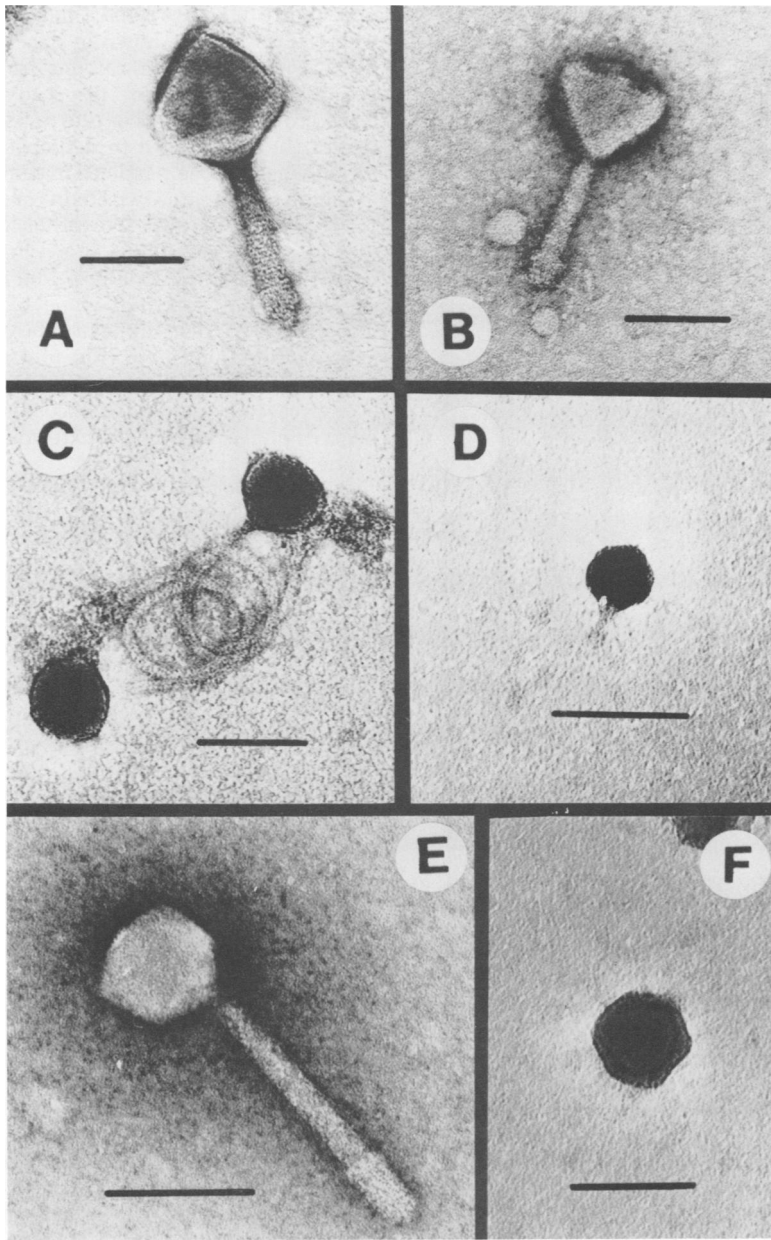


FIG. 1. Morphology of bacteriophages found as free particles in seawater of Yaquina Bay, Oregon. Bar, 0.1  $\mu\text{m}$ .

have typical bacteriophage morphologies, except for the hexagon-shaped particle in Fig. 1. This hexagon-shaped particle was observed frequently in the bay and resembles the nontailed phage described by Hidaka (8) for a marine *Pseudomonas*, but the possibility exists that it could be an animal virus. The virus (diameter, 80 nm) appears to be too large to be a member

of the group of small phages (diameters, 20 to 30 nm) that includes  $\phi\text{X174}$ , MS2, and others (15). The bacteriophage described by Hidaka and Ichida (10) is 60 nm in diameter. Figures 1A and B show bacteriophages with octahedral heads, and Fig. 1C shows two bacteriophages with long flexible tails coiled around themselves. The phage in Fig. 1D fits into group III of Tikho-

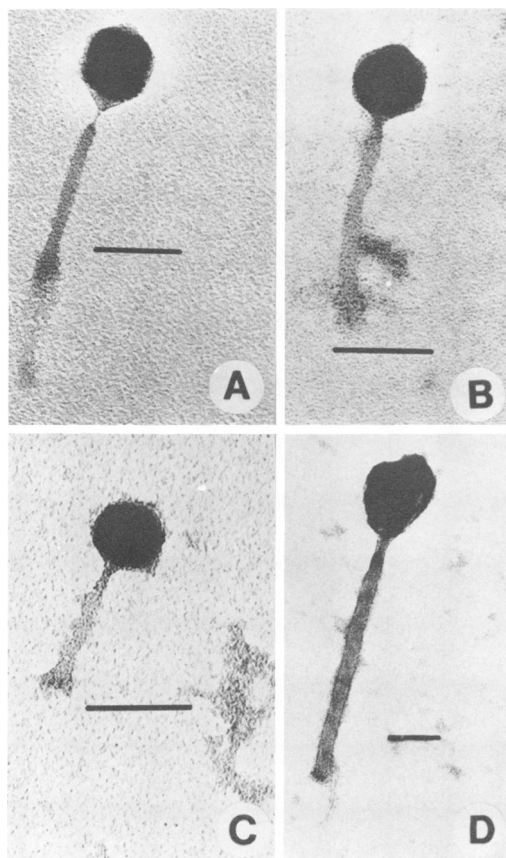


FIG. 2. Different types of bacteriophages found as free particles in seawater of Yaquina Bay, Oregon. Bar, 0.2  $\mu$ m.

nenko (15), which contains phages with short tails. Figures 1A, B, and E and 2A through D show phages with tails that could be included in type V in the classification of Tikhonenko (15). Based on the classification of Bradley (5), all of the bacteriophages with tails shown in the various figures would presumably possess double-stranded deoxyribonucleic acid. Whether the particles without tails possess single-stranded deoxyribonucleic acid or single-stranded ribonucleic acid cannot be determined from the electron micrographs.

Bacteria with bacteriophages attached to them were found readily (Fig. 3 and 4). In some cases hexagon-shaped structures attached to bacteria could be readily identified (Fig. 4C). The ability to demonstrate these types in electron micrographs reinforces the concept of the widespread occurrence of bacteriophages in the seawater of the bay.

The most frequent size of the bacteriophage heads observed in this investigation was 70 to 85

nm. Figure 1D illustrates a head size of 50 nm, whereas the heads shown in Fig. 1A and B and Fig. 4 were very large, ranging from 100 to 130 nm. The head size in this study is probably biased toward larger sizes due to the 0.2- $\mu$ m pore size of the filters used to demonstrate the bacteriophages as free particles in seawater.

Figure 5 illustrates two chains of viral particles 70 to 80 nm in diameter, together with the ghost of a bacterial cell. Figure 6 is a higher magnification of the same structures and clearly shows the hexagonal shape of some of the viruses. These virus particles appear to be covered by an extramembranous layer (Fig. 6). Certain animal viruses are surrounded by a lipid layer, and there is one case of a nontailed icosahedral marine bacteriophage having an external lipid membrane (6).

Bacteriophages are lytic agents of microbial populations, but their role in the marine environment has not yet been fully assessed. Other investigators (3, 4, 9) have argued that genetic interchange, carried out by bacteriophages between different strains of bacteria by occasional cross-infection, is theoretically possible. Tem-

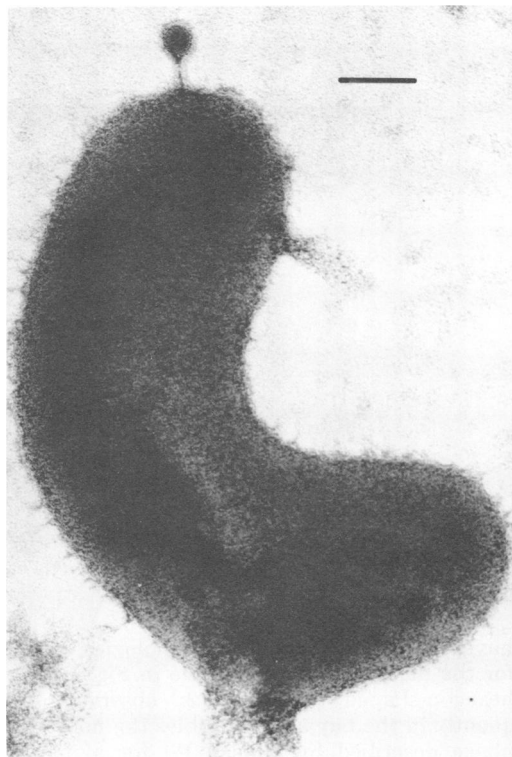


FIG. 3. Bacteriophage from seawater of Yaquina Bay, Oregon, attached to a nonflagellated vibrio-shaped bacterium. Bar, 0.2  $\mu$ m.

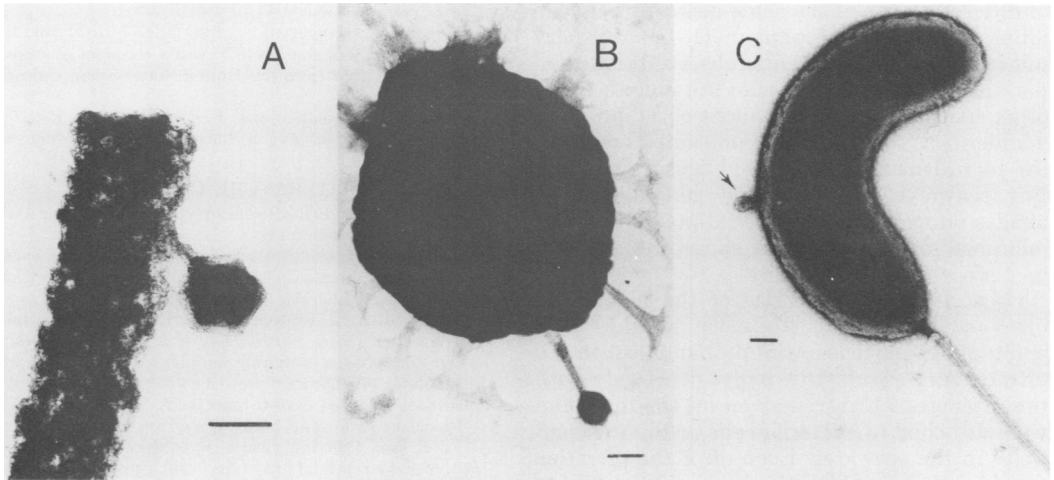


FIG. 4. (A) Phage from seawater of Yaquina Bay, Oregon, attached to a piece of cellular debris or other particulate matter. Bar, 0.1  $\mu\text{m}$ . (B) Bacteriophage from seawater of Yaquina Bay, Oregon, attached to a plasmolyzed bacterial cell. Bar, 0.01  $\mu\text{m}$ . (C) Hexagon-shaped particle (arrow) from seawater of Yaquina Bay, Oregon, attached to a flagellated vibrio cell. Bar, 0.1  $\mu\text{m}$ .

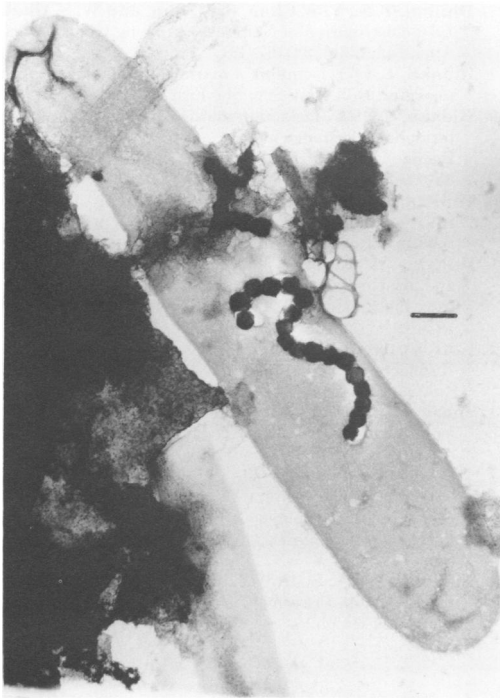


FIG. 5. Chains of hexagon-shaped viral particles from seawater of Yaquina Bay, Oregon, lying on the ghost of a bacterial cell. Bar, 0.1  $\mu\text{m}$ .

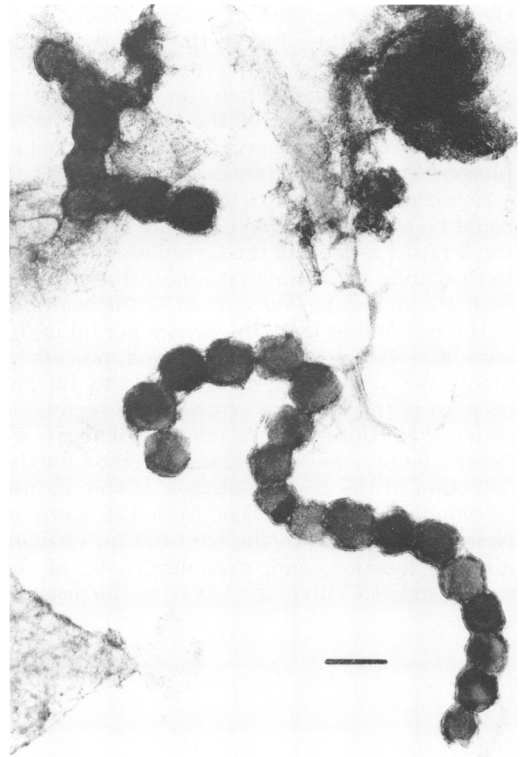


FIG. 6. Detailed magnification of Fig. 5 showing the hexagon-shaped particles resembling icosahedral viruses. The particles are surrounded by a membranous structure that seems to hold them together. Bar, 0.1  $\mu\text{m}$ .

perate bacteriophages can transfer material, including genetic material other than the viral genes themselves (2). Cross-infections by phages

of different hosts of the same genus or between different genera are common (1, 13). In dairy microbiology it is frequently observed that temperate phages for one species are able to infect other species either in a virulent or a temperate manner (11). Since the data presented point out the high density of bacteriophages in Yaquina Bay seawater, the lytic and genetic role of phages on the natural bacterial population and microbial taxonomy in near-shore water cannot be disregarded in the future.

Because of the pore size of the membrane filter employed in this study, we recognize that many phage particles were probably lost in the filtrate. As a result, this paper deals only with those phages which remained on the filter and were attached to bacterial cells or to other particles in the seawater. Even after the filtration process, the number of phage particles that could be counted on the electron microscope grids is clear evidence of the high number of bacteriophages that exist in the waters of Yaquina Bay.

It was not possible to make precise calculations as to the number of phages in any given sample due to their loss in the filtrate and the attachment of phages to bacterial cells or other particulate material. When the latter situation occurred, the density of the stained particles could obstruct the observation of any attached phages. In certain instances, however, the bacteriophages attached to a bacterium or a particle could be clearly discerned. Taking into account the limiting aspects of the techniques employed in this study, we estimated concentrations of at least  $10^3$  viruses per ml in most of the seawater of the bay and at least  $10^4$  viruses per ml in the enriched waters near the seafood processing plant. We believe these estimates to be low because of the inherent errors in the technique employed in this study. As mentioned above, we did not employ any enrichment method for the detection of the bacteriophages. It should also be noted that the sewage from the town of Newport (adjacent to the bay) has an offshore disposal location, and, therefore, none of the sewage directly enters the bay from the disposal system.

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